

Comparative Analysis of Nitrogen Assimilation Pathways in *Pseudomonas* using Hypergraphs

Aziz Mithani, Arantza Rico[†], Rachel Jones[†],
Gail Preston[†] and Jotun Hein
mithani@stats.ox.ac.uk

Department of Statistics and [†]Department of Plant Sciences,
University of Oxford, South Parks Road, Oxford, UK

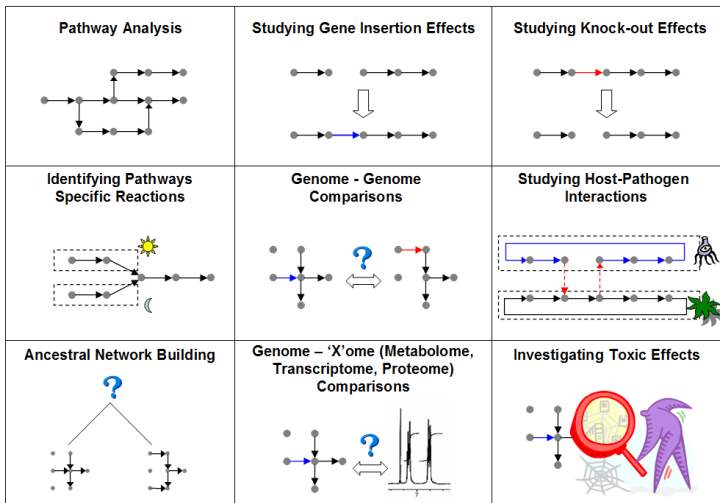
Nitrogen2007 Symposium
30 July 2007



Motivation

Metabolic networks have diverse applications . . .

Metabolic networks can be used in many different types of studies



Problem

Commonly used representation is not adequate . . .

Metabolic networks are generally represented by directed graphs

- Nodes - metabolites
- Edges - reactions / enzymes

Limitations

- Cannot capture relationship between more than two metabolites in a reaction
- Ignores dependence between the metabolites
- Not ideal for representing multiple connections (reactions) between metabolites

Solution

- Use hypergraphs

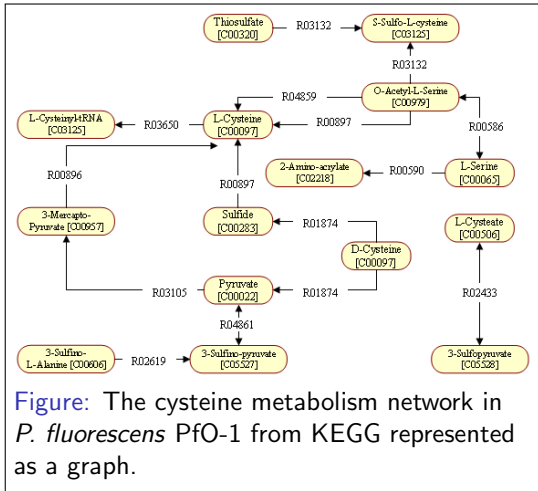


Figure: The cysteine metabolism network in *P. fluorescens* PfO-1 from KEGG represented as a graph.

Outline

The way forward ...

- 1 Hypergraph
- 2 Rahnuma: Pathway Analysis and Network Comparison Tool
 - Pathway Analysis
 - Network Comparison
- 3 Case Study: Predicting amino acid assimilation pathways
- 4 Future Work
- 5 References and Acknowledgements

Why *Pseudomonas*?

- Important pathogenic bacteria
- Occupy diverse niche
- Nitrogen assimilation is widely studied in *Pseudomonas* species



Figure: *P. aeruginosa*



Figure: Pith necrosis caused by *P. fluorescens* (Source: Saygili et al., 2004)



Figure: Halo blight of beans, caused by *P. syringae* pv. *phaseolicola*. (Source: The American Phytopathological Society)

Rahnuma: Pathway Analysis & Network Comparison Tool

A hypergraph based tool ...

Rahnuma – someone who guides through the path (Urdu language)

The Tool

- Written in Java
- Represents networks as directed hypergraphs
- Uses advanced data structures to efficiently compute the pathways between the metabolites or **group of metabolites**
- **Outperforms KEGG's PathComp tool (Kanehisa, 2006) in many areas**
 - See poster (P46) for details

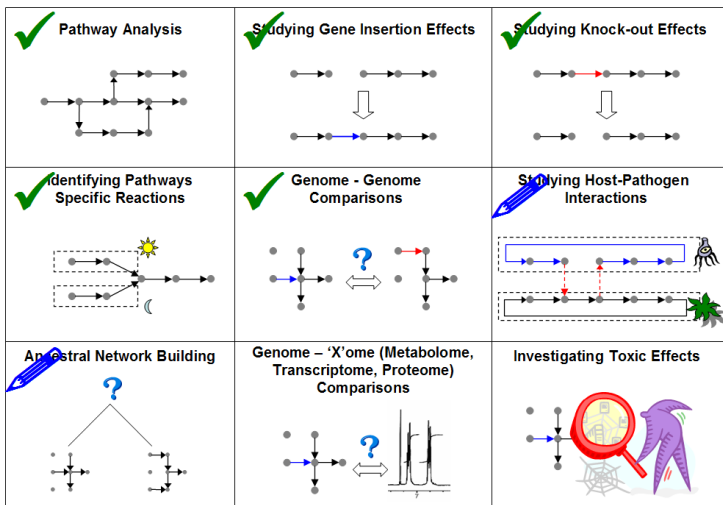
Modules

- 1 Pathway prediction and analysis
- 2 Network comparison

Rahnuma: Application Areas

Rahnuma is a versatile tool ...

Rahnuma can be used in many different types of studies



Rahnuma – Pathway Prediction and Analysis Module

Explores all possible pathways between metabolites ...

Basic Mode

- At each step of the pathway calculation, all the reactions in which the metabolite being considered is a substrate are processed
- Two types of analysis
 - 1 Connection based – Requires connection file
 - 2 Reaction based – Process all possible connections
- Optional parameters in Reaction based analysis:
 - 1 Compounds to be ignored, e.g. ATP, AMP, H₂O etc.
 - 2 Elements to be traced, e.g. carbon, nitrogen etc.

Extended Mode

- Allows following additional functionalities

Type	Annotation	in silico Experiment
P	Predicted Reaction	Gene Insertion
KO	Invalid Reaction	Knock-out

Results for Pathway Analysis

Rahnuma helps explaining the experimental results ...

Minimum predicted pathway lengths to ammonia from selected amino acids are shown below along with the experimental results (Rico and Preston, submitted).

	<i>P. fluorescens</i> PfO-1		<i>P. syringae</i> DC3000	
	Min Length	Biolog	Min Length	Biolog
Alanine	3	+	4	-/w
Arginine	1	+	2	+
Cysteine	4	-/w	5	-/w
Glutamine	1	+	1	+
Phenylalanine	1	+	3	w
Serine	1	+	1	+
Tryptophan*	2	-/w	2	-/w

*Tryptophan Pathway involves reaction R02722, a tryptophan synthesis reaction, catalysed by the enzyme tryptophan synthase (EC: 4.2.1.20). However, due to reversible nature of the reaction it may proceed in the reverse direction.

General Result

Amino acids that are rapidly assimilated as carbon or nitrogen sources have short pathways between the amino acid and the TCA cycle or ammonia.

Rahnuma – Network Comparison Module

Allows highlighting pathway specific differences between networks . . .

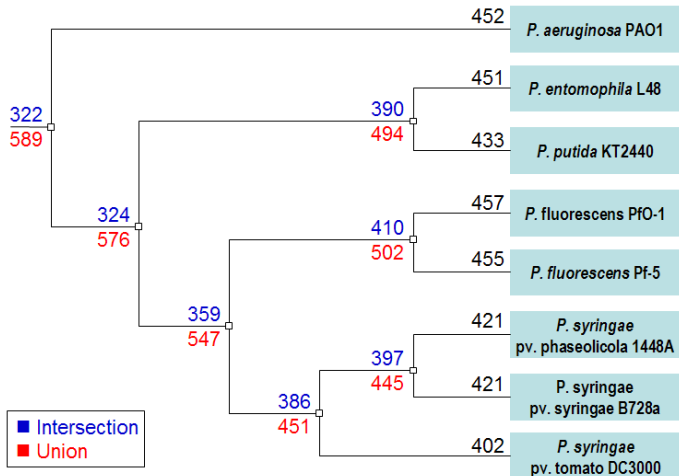
- Comparison between two networks or groups of networks
- Provides flexibility for comparative analysis at various levels
 - strain
 - species
 - . . .
- Two types of comparison
 - 1 Pathway Based
 - Extension of pathway analysis
 - Only reactions involved in the pathways from a metabolite to one or more metabolites are considered
 - Can be used to study effects of *in silico* gene insertion or knock-out
 - 2 Full Network
 - All the reactions present in the network are considered
- Output Mode
 - 1 Pathway
 - 2 Reaction

Comparative analysis on a phylogeny

Comparing networks on a phylogeny may provide clue about ancestral networks ...

Number of reaction at various levels of *Pseudomonas* phylogeny

- Includes reactions involved in amino acid assimilation and related pathways only



Case Study: Predicting Amino Acid Assimilation Pathways

Alanine Assimilation Pathways in *P. fluorescens* PfO-1

Observation: Alanine is rapidly assimilated as a carbon and nitrogen source in *P. fluorescens* PfO-1.

Hypothesis: Alanine has short pathways to the TCA cycle and ammonia.

Pathway Prediction and Analysis

	TCA cycle			Ammonia		
	$n=1$	$n=2$	$n=3$	$n=1$	$n=2$	$n=3$
KEGG's PathComp Tool [†]	✗	✗	✗	✗	✗	✗
Rahnuma [‡]	✗	✓	✓	✗	✗	✓

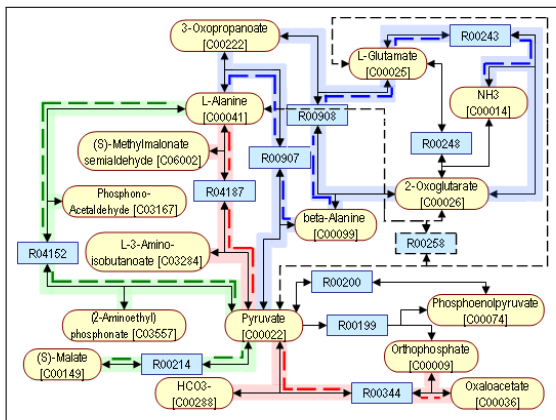
[†]Pathways calculated separately for each metabolite in the TCA cycle

[‡]Pathways calculated using Rahnuma's inherent functionality of specifying multiple destination metabolites

Case Study: Predicting Amino Acid Assimilation Pathways

Reactions reported by Rahnuma

- Subset of Reactions shown in the figure.
- Possible routes marked for carbon (red and green) and nitrogen (blue).
- None of these reactions picked by PathComp.



Comparative Analysis

- Based on KEGG's reference network
- Possibly missing reaction R00258 (marked in black) in *P. fluorescens* PfO-1 corresponding to an aminotransferase
- A shorter pathway of length 2 from alanine to ammonia

Future Work

Rahnuma has lots of potential ...

- Ancestral Network Building
- Host-pathogen interactions
- Phylogeny based analysis
- Enzyme based predictions and analysis
- Probabilistic network building
- Likelihood based prediction of pathways / reactions

References and Acknowledgements

With thanks to . . .

References

- 1 Kanehisa, M., Goto, S., Hattori, M., Aoki-Kinoshita, K.F., Itoh, M., Kawashima, S., Katayama, T., Araki, M., and Hirakawa, M. *From genomics to chemical genomics: new developments in KEGG*. Nucleic Acids Res. 34, D354-357 (2006).
- 2 Rico, A., and Preston, G. M. Apoplast phenoarrays: An integrated approach to study nutritional specialization of *Pseudomonas syringae* in the plant apoplast (submitted)

Acknowledgements



Higher Education Commission,
Government of Pakistan



St. Anne's College,
University of Oxford

The Team

and many more . . .

